HIGH PRODUCTION VOLUME CHALLENGE PROGRAM

TEST PLAN FOR

2-PROPENAMIDE, N-(1,1,3,3-TETRAMETHYLBUTYL)-

CAS # 4223-03-4

Submitted by

National Starch and Chemical Company
10 Finderne Avenue
Bridgewater
NJ 08807

A member of the ICI Group

CONTENTS

1 INTRODUCTION

1.1 Submission details

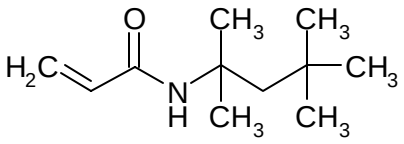
As part of the ICI group and on behalf of ICI Americas Inc, National Starch and Chemical Company are sponsoring 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- under the High Production Volume (HPV) Challenge Program. This document summarizes the available data and outlines the test plan designed to meet the requirements of the HPV challenge program.

1.2 General Substance Information

1.2.1 Identity and synonyms

CAS Name:	Acrylamide, *N*-(1,1,3,3-tetramethylbutyl)-
IUPAC Name:	*N*-(1,1,3,3-tetramethylbutyl)acrylamide
Common name:	2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- used in this summary
Other names:	*tert*-Octylacrylamide; t-OAA TOA
CAS number:	4223-03-4
Molecular weight:	183.3
Molecular formula:	$C_{11}H_{21}NO$
SMILES Code:	O=C(NC(CC(C)(C)C)(C)C)C=C

1.2.2 Chemical structure



1.3 Use

2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-, when polymerized with a variety of other vinyl or acrylic monomers, is used to produce a wide range of polymers which find use as ingredients in the personal care and adhesives industry. Typical applications in which 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-containing polymers are used include hairsprays, gels, mousse, skin care products, medical tapes and transdermal drug-delivery systems. Since there are no consumer uses of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- in its non-polymerized form, exposure to the chemical substance in consumer products is minimal.

1.4 Manufacturing

2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is produced by the addition of acrylonitrile and diisobutylene in an acidic environment. The final reaction product is a waxy solid that may be ground into a flake or dissolved in an appropriate solvent for use in the liquid state. Final product in flake form is packaged in sealed drums for storage or offsite shipment. For onsite use, the flake material is dissolved in an appropriate solvent in a mixing tank then piped to storage tanks for later use. Onsite storage and delivery of liquid 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is carried out within a closed piping system to minimize potential exposure to the substance.

1.5 Experiences with Human Exposure

Human exposure is minimal throughout the manufacture of 2-propenamide, *N*-(1,1,3,3-tetramethylbutyl)-. Production is carried out primarily in a closed system. Potential worker exposure may occur during sampling processes and again during filtration, grinding, blending and drum-off processes. These processes generally involve only one or two individuals for short periods of time. Maintenance line openings of the 2-propenamide, *N*-(1,1,3,3-tetramethylbutyl)- storage and delivery system are extremely rare, thus minimizing another potential source of worker exposure. A study conducted to examine percutaneous absorption of 2-propenamide, *N*-(1,1,3,3-tetramethylbutyl)- demonstrated low potential for dermal penetration of this chemical. 2-propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is a waxy solid with negligible vapor pressure. These properties further limit human exposure of 2-propenamide, *N*-(1,1,3,3-tetramethylbutyl)- via the dermal and inhalation routes. In all cases, appropriate PPE and engineering controls are utilized to minimize human exposure to the substance. Polymerization processes in which 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is used as a raw material are also performed in closed systems that are designed to minimize workplace exposure to the chemical substances used.

1.6 Rationale for specific categorization

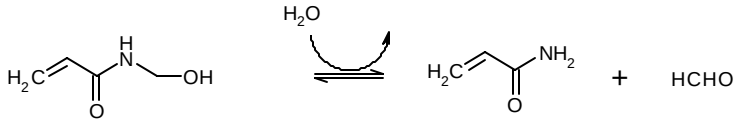
The NMA/NBMA association has sponsored the acrylamide derivatives *N*-hydroxymethylacrylamide (NMA) and *N*-butoxymethylacrylamide (NBMA) under the HPV challenge program as the N-(methyl)-acrylamide category. Although superficially similar in chemical structure to 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-, their behavior in water and genotoxicity are sufficiently different for them to be considered as a separate chemical category distinct from 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-. The rationale for this is explained below.

1.6.1 Behavior in water

NMA and NMBA hydrolyze according to the following reaction sequences.[1]

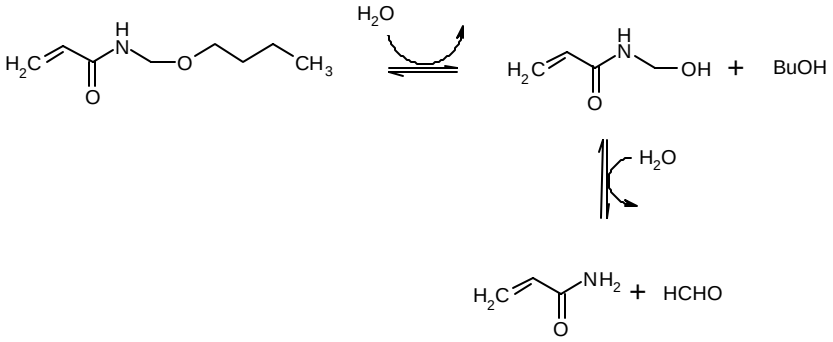
1.6.1.1 NMA

Dilute aqueous solutions of NMA are unstable at neutral pH conditions and undergo hydrolysis to yield acrylamide and formaldehyde.



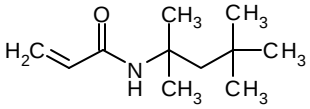
1.6.1.2 NMBA

Dilute aqueous solutions of NBMA undergo slow hydrolysis to give a mixture of NMA, acrylamide, *n*-butanol and formaldehyde.



1.6.1.3 2-Propenamide N-(1,1,3,3-tetramethylbutyl)-

Unlike NMBA and NMA, which are *N*-substituted with a -CH_2-O-R group (where R=H, or C_4H_9), 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is alkyl substituted with a 1,1,3,3-tetramethylbutyl group and so lacks a hydrolytic site, and is expected to be more stable in water than NMA or NMBA.



1.6.2 Genotoxicity

The genotoxicity of NMA and NBMA is described in the HPV test plan and summary.[1] A comparison of their genotoxicity with 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl) and acrylamide‡ is shown in Table 1. Details of the genotoxicity of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- are described in section 2.4.3 Genetic toxicity. The data for NMA and NBMA show a mixture of positive and negative results dependent on the assay system whereas 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- gave negative results in three assay systems used.

Table 1: Comparison of genotoxicity

Endpoint	**Acrylamide‡**	**NMA**	**NBMA**	***2-Propenamide, N-(1,1,3,3-tetramethylbutyl)-***
in-vitro	Ames (w/wo* multiple tests) - negative	Ames (w/wo multiple tests) - negative	Ames (w/wo multiple tests) - negative	Ames (w/wo single test) - negative
	Chrom. Abs and SCE (CHO, wo) - positive	Chrom. Abs and SCE (CHO, w/wo) - positive	Chrom. Abs (CHO, w/wo) - positive	
	Bacterial gene mutation assay (Kleb. pneun., wo) - negative	Chrom. Abs (BALB, w/wo) - negative		
	E. coli reverse			

‡ The NMA/NBMA proposal cross referenced data on acrylamide, and this has been included for completeness.

Endpoint	Acrylamide‡	NMA	NBMA	*2-Propenamide, N-(1,1,3,3-tetramethylbutyl)-*
	mutation assay (wo) - positive			
	HPGRT (Mouse Lymphoma, w/wo) - positive			L5178Y TK Mouse Lymphoma (w/wo) - negative
	HPGRT (CHO, wo) - negative			
	UDS - positive/negative			
in-vivo	Chrom. Abs. negative/positive			
	Sex-linked Recessive lethal - negative			
	Mouse Heritable translocation - positive			
	Rodent dominant lethal - positive/ negative			
	UDS - positive			
	Micronucleus - positive	Micronucleus - negative		Micronucleus - negative
	Transgenic Mouse (multiple) - negative			

Abbreviations

w/wo With and without metabolic activation
ND Not determined.
Chrom Abs Chromosome aberration
SCE Sister Chromatid Exchange
CHO Chinese Hamster Ovary
UDS Unscheduled DNA Synthesis

1.6.3 Conclusion

The differences in the behavior in water and genotoxicity support the non-inclusion of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- in the N-(methyl)-acrylamide category.

2 TESTING PLAN AND RATIONALE

2.1 Physicochemical Properties

2.1.1 Appearance

2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is an off-white/white waxy solid.

2.1.2 Melting Point

The melting point of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- was reported as 55-60°C, no other details are available.[2]

2.1.3 Boiling Point

No measured data are available.

2.1.4 Vapor Pressure

No measured data are available.

2.1.5 Partition coefficient (*n*-octanol/water)

No measured data are available.

2.1.6 Water solubility

The water solubility was reported as <1g/L, no other details were available.[3]

2.1.7 Testing plan for physicochemistry

It is proposed to carry out melting/boiling point, water solubility, vapor pressure and partition co-efficient studies using OECD protocols.

2.2 Environmental fate and behavior

2.2.1 Photodegradation

There are no measured data available for the photodegradation of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-. The photodegradation was estimated using the AOPWIN module of EPIWIN v 3.10[4] as 7.6 hours assuming a 12 hour day and a hydroxyl concentration of $1.5 \times 10^6 cm^{-3}$. The calculation is considered to meet the data requirement.

2.2.2 Hydrolysis

There are no data available for the hydrolysis of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-. However, from structural considerations (see section 1.6.1) it is expected to be hydrolytically stable.

2.2.3 Ready biodegradation

There are no data available for the biodegradation of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-.

2.2.4 Transport/distribution between environmental compartments

There are no measured data available. The Level III fugacity module of EPIWIN v3.10[5] will be used to determine the relative distribution of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- between air, water, soil and sediment once reliable physicochemical data are available. The calculation is considered to meet the data requirement.

2.2.5 Testing plan for environmental fate and behavior

The estimation of photodegradation is considered to satisfy this endpoint and no further testing is proposed. In the absence of hydrolysis and ready biodegradation data, new studies using OECD protocols will be commissioned. Transport/distribution between environmental compartments will be addressed by calculation using the EPIWIN model with the measured physicochemical inputs.

2.3 Environmental Toxicology

2.3.1 Acute toxicity to fish

No measured data are available.

2.3.2 Acute toxicity to daphnia

No measured data are available.

2.3.3 Toxicity to algae

No measured data are available.

2.3.4 Summary of environmental toxicology and test plan

It is suggested that testing of the physical chemical properties and the fate and behaviour in the environment are carried out prior to a decision on the conduct of testing in fish, daphina and algae. These tests will provide information on the environmental compartment of interest and may allow the waiver of testing, at least for testing in fish species. Should any or all of the tests be required once more data have been generated on the test material, then National Starch and Chemical Company are committed to conducting the testing.

2.4 Mammalian Toxicology

2.4.1 Acute toxicity

No data are available.

2.4.2 Repeated-dose toxicity

No data are available.

2.4.3 Genetic toxicity

2.4.3.1 Gene mutation

2.4.3.1.1 *In-vitro* bacterial (Ames) assay

The mutagenicity of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- was examined by incubating this chemical with *S. typhimurium* (TA98, TA100, TA1535, or TA1537) or *E. coli* (WP2uvrA). Increasing doses ranging from 33.3 to 5000 µg/plate were dissolved in dimethylsulfoxide with or without Aroclor™-induced rat liver (S9) mix and incubated for 52 hours at 37°C. In the initial and confirmatory assays, 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- did not cause a positive increase in the number of revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes. Under the conditions of the assay, 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- was not mutagenic in the tested bacteria strains. The study was conducted to GLP and in accordance with OECD method 471. [6]

2.4.3.1.2 *In-vitro* mammalian gene mutation assay

The mutagenicity of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- was examined in mammalian cells by incubating increasing concentrations of the above chemical with L5178Y *TK* Mouse Lymphoma cells for four hours at 37°C. The vehicle was dimethylsulfoxide. Due to cytotoxicity, the range of concentrations varied for those incubated with Aroclor™-induced rat liver (S9) mix. The concentrations of 2-Propenamide,

N-(1,1,3,3-tetramethylbutyl)- were 50 to 600 µg/ml without activation and 25 to 500 µg/ml with activation in an initial and confirmatory assay. Cytotoxicity was induced at the highest concentrations in both trials. Colony sizing was carried out for the test substance and positive and vehicle controls. None of the analyzed treatments in either trial induced an increase in mutant frequency or change in colony size. The positive controls produced the expected response. 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-, was not mutagenic under the conditions of the L5178Y *TK* Mouse Lymphoma Forward Mutation Assay. The study was conducted to GLP and in accordance with OECD method 476.[7]

2.4.3.2 Chromosome aberration

2.4.3.2.1 *In-vivo* micronucleus assay

The genotoxicity of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- was investigated in a mouse micronucleus study. Both sexes responded similarly in preliminary studies. Thus only males were used in the main study. Increasing doses of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- (corn oil vehicle, 175, 300 or 700 mg/kg) were administered orally to groups of six male CD-1 mice. Bone marrow was harvested at 24 hours (all doses) and 48 hours (control and 700 mg/kg). The polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) ratio and the number of micronucleated PCEs were determined. The test article induced signs of clinical toxicity in the treated animals and was cytotoxic to the bone marrow indicated by a significant decrease in the PCE:NCE ratio in the 700 mg/kg group at the 48 hour harvest time point. No change in micronucleated PCEs was observed at any dose level or harvest time point. The positive control, cyclophosphamide produced the expected increase in micronucleated PCEs. 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- was not clastogenic under the conditions of the study. The study was conducted to GLP and in accordance with OECD method 474.[8]

2.4.3.3 Genetic toxicity test plan and summary

Bacteriological mutagenicity (Ames), *in-vitro* mammalian cell gene mutation and *in-vivo* mouse micronucleus assays were negative. The guideline requires that the endpoints for gene-mutation and chromosome aberration are addressed. The bacteriological mutagenicity (Ames) and mammalian gene mutation assays meet the requirements for this endpoint. The second endpoint, identification of chromosome alterations is met with the *in vitro* mammalian gene mutation and the mouse micronucleus assays. The mouse lymphoma gene mutation assay can identify clastogenicity by differentiating between small and large colony sizes. In this assay, small colonies are indicative of chromosome damage and large colonies gene mutation[9]. Furthermore, the *in-vivo* mouse micronucleus assay detects chromosome damage. In view of the negative findings in these assays demonstrating that 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is neither mutagenic or clastogenic, no further testing is required for this endpoint. The results of the genotoxicity testing of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- are summarized in Table 2.

Table 2: Genetic toxicity summary and test plan

End point	Method	GLP, year	Outcome	Further testing required	Reliability
Gene mutation					
In-vitro Bacterial gene mutation assay(2.4.3.1.1)	OECD 471	Yes, 1998	Negative	No	Reliable without restrictions (1)
In-vitro Mammalian gene mutation assay(2.4.3.1.2)	OECD 476	Yes, 1998	Negative	No	Reliable without restrictions (1)

End point	Method	GLP, year	Outcome	Further testing required	Reliability
Chromosome aberration					
In-vitro Mammalian gene mutation assay(2.4.3.1.2)	OECD 476	Yes, 1998	Negative	No	Reliable without restrictions (1)
In-vivo Micronucleus assay(2.4.3.2.1)	OECD 474	Yes, 1998	Negative	No	Reliable without restrictions (1)

2.4.4 Reproductive toxicity

No data are available.

2.4.5 Fertility

No data are available.

2.4.6 Mammalian toxicology test plan

A combined repeat dose/reproduction/developmental study (OECD 422) will be carried out to adress data-points 2.4.2 Repeated-dose toxicity, 2.4.4 Reproductive toxicity and 2.4.5 Fertility. It is proposed that an acute study is not required as signs of clinical toxicity were detected in the existing single dose in vivo genetic toxicity study. Data from this study will therefore be used to assist in the dose selection for the developmental study, together with a dose range-finding study. This approach will ensure the minimum numbers of animal are sacrificed to fulfill the data requirements.

2.5 Additional Data

2.5.1 *In-vitro* dermal absorption

The dermal absorption of 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- was investigated in an *in-vitro* rat and human percutaneous absorption assay using a glass diffusion assay design based on the then current draft OECD protocol.[10] This study was conducted in accordance with GLP. The integrity of the epidermal membranes was confirmed by measurement of electrical resistance. The 10 mg/cm^2 of test material was applied to 6 replicates, each, of rat and human epidermal membranes and incubated unoccluded for up to 24 hours. The concentration of test chemical in the 50% aqueous ethanol receptor fluid was sampled at 6, 8, 10 and 24 hours after dosing and determined by gas-liquid chromatography. For human epidermis, the amounts absorbed at less than ten hours were at or below the limit of quantification (5 µg/cm^2) increasing to a maximum of 9.4 µg/cm^2 at 24 hours. Over the 6-24 hour exposure period, the mean absorption rate was 0.522 µg/cm^2/hr. The mass balance mean percentage recovered was 90%. Most of the dose, 85.7% (mean percentage) was recovered by mild skin washing, whereas 0.1% was detected in the epidermal membrane. For rat epidermis, the mean absorption rate was 1.386 µg/cm^2/hr. The mass balance mean percentage recovered was 90.6%. Again, most of the dose, 90.6% (mean percentage) was recovered by mild skin washing but no chemical was recovered from the epidermal membrane. 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is considered to have a low rate of dermal penetration.[11]

2.6 Summary of Test Plan

The test plan for 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)- is summarized below in Table 3.

Table 3: Overall test plan for 2-Propenamide, *N*-(1,1,3,3-tetramethylbutyl)-

Data Point		Data Available	Test Planned	Protocol
PHYSICOCHEMISTRY				
2.1.2	Melting Point	Y	Y	OECD 102
2.1.3	Boiling Point	N	Y	OECD 103
2.1.4	Vapor Pressure	N	Y	OECD 104
2.1.5	Partition coefficient (n-octanol/water)	N	Y	OECD 117
2.1.6	Water solubility	Y	Y	OECD 105
ENVIRONMENTAL FATE AND BEHAVIOR				
2.2.1	Photodegradation	Y	N	Not applicable
2.2.2	Hydrolysis	N	Y	OECD 111
2.2.3	Ready biodegradation	N	Y	OECD 301B
2.2.4	Transport/distribution between environmental compartments	N	Calculation[5]	Not applicable
ECOTOXICOLOGY				
2.3.1	Acute toxicity to fish	N	Y*	OECD 203
2.3.2	Acute toxicity to daphnia	N	Y*	OECD 202
2.3.3	Toxicity to algae	N	Y*	OECD 201
MAMMALIAN TOXICOLOGY				
2.4.1	Acute toxicity	N	N	Use of in vivo genetic tox data and range-finding data
2.4.2	Repeated-dose toxicity	N	Y	OECD 422
2.4.3.1	Gene mutation	Y	N	Not applicable
2.4.3.2	Chromosome aberration	Y	N	Not applicable
2.4.4	Reproductive toxicity	N	Y	OECD 422
2.4.5	Fertility	N	Y	OECD 422

* Testing strategy to be confirmed once physical chemical and environmental fate data are available

3 REFERENCES

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